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Minireview

Current Approaches for Absorption, Distribution, Metabolism, and Excretion Characterization of Antibody-Drug Conjugates: An Industry White Paper

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ABSTRACT

An antibody-drug conjugate (ADC) is a unique therapeutic modality composed of a highly potent drug molecule conjugated to a monoclonal antibody. As the number of ADCs in various stages of nonclinical and clinical development has been increasing, pharmaceutical companies have been exploring diverse approaches to understanding the disposition of ADCs. To identify the key absorption, distribution, metabolism, and excretion (ADME) issues worth examining when developing an ADC and to find optimal scientifically based approaches to evaluate ADC ADME, the International Consortium for Innovation and Quality in Pharmaceutical Development launched an ADC ADME working group in early 2014. This white paper contains observations from the working group and provides an initial framework on issues and approaches to consider when evaluating the ADME of ADCs.

Introduction

Antibody-drug conjugates (ADCs) are novel molecular entities that leverage the specificity of a monoclonal antibody (mAb) to deliver a potent drug to the intended pharmacological target to achieve the desired therapeutic effect. An ADC is composed of a drug molecule conjugated to a mAb via a linker (Fig. 1). ADCs are mostly used in oncology, where they provide targeted delivery of the cytotoxic drug and thus broaden its therapeutic margins. The most frequently employed mAbs in the clinic are of the IgG1 isotype (Deslandes, 2014), and the same is true for mAbs used in ADCs. Several classes of drugs are currently being used for ADCs. The most advanced of them are microtubule-disrupting agents, such as auristatins like monomethyl auristatin E (MMAE) and monomethyl auristatin F; maytansine derivatives (DMs), such as DM1 and DM4; and DNA-damaging agents, such as calicheamicins and duocarmycins (Adair et al., 2012). The conjugation of these drugs to mAbs is typically achieved via the ε-amino group of lysines or the thiol residue of cysteines (reduced interchain disulfides or genetically engineered cysteines) on the antibody molecule and a chemical linker. The most frequently used linkers are noncleavable alkyl linkers, such as *N*-maleimidomethylcyclohexane-1-carboxylate (used in Kadcyla); enzymatically cleavable linkers, such

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ABBREVIATIONS: ADA, antidrug antibody; ADC, antibody-drug conjugate; ADME, absorption, distribution, metabolism, and excretion; DAR, drug/antibody ratio; DDI, drug–drug interaction; DM, maytansine derivative; mAb, monoclonal antibody; MMAE, monomethyl auristatin E; P450, cytochrome P450; PK, pharmacokinetics; Tab, total antibody.

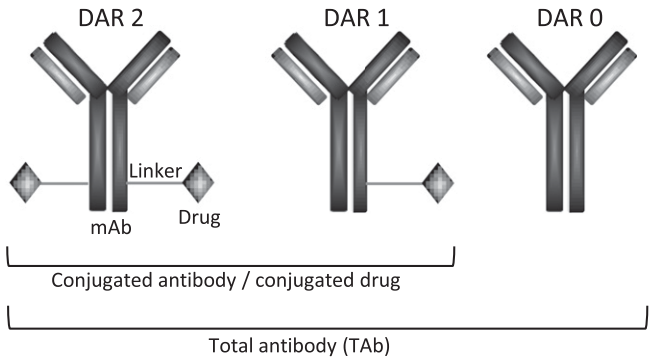

Fig. 1. Diagram of a typical ADC. An ADC consists of a mAb conjugated to a drug via a linker. Different numbers of drug molecules can be attached to a single mAb, which results in ADCs with different DARs.

as the self-immolative para-aminobenzyl group attached to a cathepsin-labile valine-citrulline dipeptide (used in Adcetris); and acid-labile hydrazone linkers (used in Mylotarg) (Sapra et al., 2011). Depending on the conjugation chemistry used, different numbers of drug molecules can be attached to a single mAb. The differences in the drug/antibody ratio (DAR) can affect ADC distribution and pharmacokinetics (PK) (Lyon et al., 2015). Each of the individual components of an ADC molecule contributes to the complexity of its disposition and overall absorption, distribution, metabolism, and excretion (ADME) properties. To simplify the terminology, this review refers to the components of an ADC as outlined in Table 1.

The mechanism of action of an intact ADC involves binding of the mAb to its target antigen on a cell surface, followed by internalization via receptor-mediated endocytosis, trafficking from endosomes to lysosomes, and intracellular release of the drug (Fig. 2) (Alley et al., 2010; Sapra et al., 2011; Adair et al., 2012). In addition, ADCs can also be taken up nonspecifically via pinocytosis into cells that do not express their target antigen, which may contribute to efficacy in the tumor environment or potentially result in adverse effects in normal tissues. The release of the drug from the ADC in the cell could occur via proteolysis of the linker, as with a cleavable linker, or by catabolism of the entire ADC, as with a noncleavable linker (Alley et al., 2010; Sapra et al., 2011; Adair et al., 2012). Alternatively, internalized ADCs could be recycled back into the circulation via the neonatal Fc receptor–mediated process, a pathway well characterized for mAb-based therapies (Roopenian and Akilesh, 2007). In general, it is desirable that the ADC molecule is stable in the circulation and drug is released only in the target tissue. To minimize systemic toxicity from the released drug, it would need to be rapidly cleared, preferably via several orthogonal pathways, to minimize potential for drug–drug interactions (DDIs) and toxicity.

To fully assess the ADME of a novel ADC, one has to characterize the disposition of the intact molecule as well as its components: the target-mediated and catabolic clearance of the mAb, the release, and traditional small molecule distribution, metabolism, and excretion of the released drug. This characterization is important during candidate optimization and development. It can facilitate rational drug design, selection of the appropriate nonclinical models, and prediction of ADME properties in the clinic.

With an increase in the number of ADCs at various stages of nonclinical and clinical development, pharmaceutical companies have been exploring diverse approaches for ADC ADME characterization. To identify the key issues worth examining when developing an ADC and find the most optimal experimental systems, the International Consortium for Innovation and Quality in Pharmaceutical Development (referred to hereafter as the IQ Consortium) launched an ADC ADME working group in early 2014. The IQ Consortium is an organization of pharmaceutical and biotechnology companies providing a forum to address issues for the biopharmaceutical industry. This white paper contains observations from the working group and provides an initial framework on issues and approaches to consider when evaluating the ADME of ADCs. However, there needs to be a continuous re-evaluation of ADME approaches as ADC technology evolves and matures over the next several years.

Overview of ADC Bioanalysis

A typical ADC is a heterogeneous mixture containing multiple drug molecules attached to an antibody at different DARs. Because of their unique composition, heterogeneous nature, and ability to undergo further dynamic changes in vivo, multiple bioanalytical methods are developed for the characterization of ADCs. These methods rely on techniques used for biologics and small molecule drugs, but there are also novel methods developed specifically for ADCs such as DAR determination (Xu et al., 2013; Hengel et al., 2014). A comprehensive description of the most important ADC analytes and details on the toolbox of bioanalytical techniques was recently published in special issues of *Bioanalysis* (Gorovits et al., 2013; Kaur et al., 2013; Gorovits 2015; Kumar et al., 2015; Myler et al., 2015; Saad et al., 2015); thus, it is not the intent of this article to discuss them. ADCs could be considered as "prodrugs" because the small molecule drug has to be released from the ADC to exert its effect. Therefore, measuring the PK of the ADC and released drug serves to confirm the mechanism of drug release and helps uncover the ADME pathways important in ADC disposition. Once the clearance mechanisms and the relationship between the different drug-containing products have been established, the ultimate goal is to identify the active species that drive efficacy and toxicity. Because of the limited clinical experience with ADCs, this has not yet been well delineated; therefore, multiple analytes are commonly measured (Fig. 1). Typically these are the total antibody (TAb; includes conjugated, partially conjugated, and unconjugated antibody; i.e., DAR $\geq$ 0), ADC (conjugated and partially conjugated antibody; i.e., DAR $\geq$ 1) or antibody-conjugated drug (total drug conjugated to antibody), and unconjugated drug (small molecule drug released from the antibody).

The need to simultaneously optimize multiple components of an ADC (drug, linker, and mAb) represents a challenge that could be assisted by the application of mathematical modeling and simulation (Singh et al., 2015). Modeling and simulation can be utilized to identify which of the analyte(s) is critical for establishing exposure-response relationships for both efficacy and safety to reduce, on a case-by case basis, the number of bioanalytical methods necessary to characterize exposure in later stage clinical development. In addition, PK/pharmacodynamic models incorporating DAR information could describe the relative contributions of the

TABLE 1

Terminology used in this review

Term	Definition
Drug	Also referred to as "payload," "warhead," or "toxin." Compound that exerts the intended pharmacological effect (e.g., tubulin binding) of an ADC. In the case of a cleavable linker, intact drug is released from the ADC. In the case of a noncleavable linker, released from the ADC drug contains the linker and an amino acid fragment
mAb	Antibody portion of the ADC
Linker	Chemical bridge that links the drug to the mAb
ADC	Whole antibody-drug conjugate molecule that contains the drug conjugated to the mAb via a linker

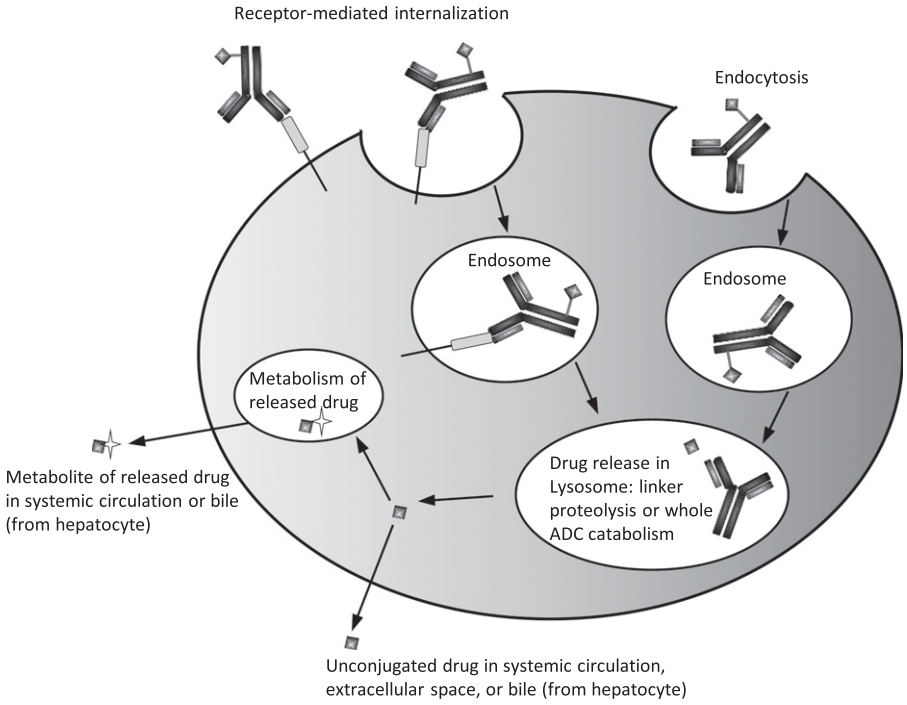

Fig. 2. Disposition of a typical ADC. An ADC can be delivered inside the cell either by antigen receptor-mediated internalization or by nonspecific endocytosis followed by the drug release in the lysosomal compartment through linker proteolysis or whole ADC catabolism. The free drug can bind to its intracellular target or be released from the cell into extracellular space or systemic circulation. In addition, in case of a cell containing drug-metabolizing enzymes, the free drug can be metabolized and metabolites can be secreted (along with the unchanged drug) into the systemic circulation or bile (in case of hepatocytes).

various drug-containing species to efficacy and toxicity and could aid in the optimization of linkers and drugs for future ADCs.

Similar to other biotherapeutics, ADCs can elicit an immune response in vivo that may alter their PK, efficacy, or safety. Based on the limited published clinical experience with ADCs thus far, risk assessment and bioanalytical strategies (namely screening, confirmatory, and neutralizing assays) followed for traditional mAbs-based therapeutics can be appropriate for ADCs (Carrasco-Triguero et al., 2013). Since antidrug antibodies (ADAs) can be generated against the mAb, the drug, or the linker portion of the ADCs, additional characterization might be necessary to determine the specificity of the ADA response. This information may help to understand potential alterations in PK and efficacy or safety of the ADC.

Disposition Mechanisms of an Intact ADC

The ADC technology is still in its infancy. Because of the very few ADCs on the market and the limited amount of available literature, the biologic and chemical processes that drive ADC disposition and thus contribute to efficacy and safety of ADCs are not fully understood. The ADME of an ADC is influenced by all three components of the molecule. The PK of an ADC is primarily driven by the carrier mAb backbone (Lin and Tibbitts, 2012). However, the linker, drug, and DAR also affect stability and PK of the ADC as a whole (Hamblett et al., 2004; Senter, 2009; Lyon et al., 2015). The complex interplay between the ADC components is a topic of intense investigation. For example, in the study conducted by Lyon et al. (2015), an anti-CD70 mAb, h1F6, was conjugated to various linkers differing in their hydrophobicity. As the hydrophobicity decreased, the clearance of the ADCs decreased, whereas there was an increase in the half-life and area under the curve. Both the distribution and elimination phases were seen to change, indicating an overall change in the disposition of the ADCs. The most hydrophilic ADC had a concentration time profile similar to that of the naked mAb, pointing to the importance of optimizing the linker. Another interesting finding was the rapid and increased hepatic uptake of an ADC, especially by the Kupffer cells, compared with the naked mAb. Uptake processes like these might explain the difference in the in vivo disposition of an ADC from that of its parent antibody and highlight the need to treat ADCs as unique entities.

An ADC can be cleared from circulation by target-mediated uptake followed by degradation in the lysosomal compartment (Fig. 2). In addition, an ADC can be subject to nonspecific uptake via pinocytosis and catabolism by certain cell types in multiple organs, including the liver, similar to traditional mAbs. Upon internalization, ADCs can be recycled by the neonatal Fc receptor, which may result in prolonged systemic exposure. In addition, deconjugation of the ADC can result in conversion of the ADC to species with different drug loads as well as unconjugated mAb. The types of in vitro and in vivo studies that could be used to characterize ADC ADME are discussed below and summarized in Table 2. In general, similar studies would be conducted for ADCs with cleavable versus noncleavable linkers, because the fundamental ADME questions that need to be answered are the same.

TABLE 2

Types of in vitro and in vivo studies for characterization of ADC ADME

Molecule	ADME Data
ADC[a]	In vitro stability in plasma or serum from animals and humans
ADC[a]	PK in pharmacology and toxicology species
ADC[b]	Animal (rodent) ADME: PK, excretion, and metabolism
ADC	Identification of circulating metabolites formed from the released drug in patients
Drug	Rodent PK
Drug	Plasma protein binding across species
Drug	In vitro characterization of metabolites formed from the released drug (safety species and human)
Drug	Reaction phenotyping
Drug	Passive/active (uptake or efflux) transport (as substrate)
Drug	P450 inhibition and induction

[a]Analytes that could be measured as appropriate include Tab, ADC, and unconjugated drug.
[b]This evaluation is recommended to be conducted with an ADC bearing a radiolabel on the drug.

ADC Stability in Systemic Circulation. Ideally, ADCs should be stable in the blood and release the drug only in the target tissue. However, since ADCs remain in the circulation for several days after their administration and are continuously exposed to plasma proteases, a gradual release of the drug is possible, depending on the nature of the linker chemistry. Plasma stability of an ADC can potentially be different across species. Understanding the mechanism and extent of drug release in the circulation can help develop ADCs with optimal safety/efficacy profiles because it is important to engineer the right balance into the ADC molecule, which would allow it to be stable in the circulation but promptly release the drug in the target cells. Instability in plasma might lead to premature release of the drug in the circulation and its subsequent distribution to tissues, potentially leading to dose-limiting toxicities (Saber and Leighton, 2015). Evaluation of in vitro stability of an ADC in plasma serves to provide information on linker stability in the systemic circulation in multiple species, as well as on potential released drug-containing products. These studies can be conducted in plasma or serum from humans as well as relevant nonclinical species (the incubation is typically conducted at 37°C at pH 7.4 for at least 96 hours at an ADC concentration around the observed or predicted C_{max} in animal species or humans). Formation of the released drug, as well as DAR changes, is usually quantified over the study duration. These studies, when conducted early in the ADC discovery process, can help optimize the combination of the mAb, linker, and the drug molecule.

Effect of DAR on ADME Properties. Depending on the conjugation chemistry, different numbers of drug molecules can be attached to a single antibody, which is characterized by the DAR representing the average number of drug molecules per antibody molecule. In addition, the DAR of an ADC may change over time in vivo. The initial DAR and rate of its change in vivo are important parameters for an ADC, because they may affect the ADC's physicochemical properties, efficacy, safety, and PK. ADCs with high DARs tend to aggregate and have higher clearance than the unconjugated mAb or lower loaded species (Hamblett et al., 2004; Senter, 2009; Lyon et al., 2015). For example, in a study conducted by Hamblett et al. (2004), SCID mice were treated with naked mAb or DAR2, DAR4, or DAR8 ADCs. The results suggested that although their half-lives were similar, the ADCs with higher DARs had lower exposures (area under the curve) and greater clearance than the naked antibody or DAR2 ADC. An examination of the concentration time profiles showed a change in the distribution phase of the three ADCs, whereas the terminal phases were parallel; this is reflected in the increasing volume of distribution with increased DAR. Although the DAR2 ADC had exposure closest to the naked mAb, it was the DAR4 ADC that had the best efficacy in the mouse xenograft model, demonstrating that optimizing the DAR for both PK and efficacy was important and not one or the other.

DAR-related ADC aggregation can also potentially change the organ uptake and mechanism of clearance of ADCs, thereby exposing the liver and/or other organs to potentially undesirable high levels of active drug. Moreover, at higher DARs, a more hydrophilic drug may have less of an effect on the disposition of the ADC than a hydrophobic drug (Lyon et al., 2015).

In addition, novel technologies, such as masking drug hydrophobicity, can be utilized to provide uniform, higher drug loads with improved PK and efficacy (Lyon et al., 2015). Since the drug can potentially be metabolized while still conjugated to the mAb, those changes can be reflected in the DAR values. In addition, the effects of site-specific versus conventional conjugation should be considered because this can affect the drug release from an ADC (Shen et al., 2012b). The DAR is typically measured by high-resolution mass spectrometry (Xu et al., 2013; Hengel et al., 2014), which can be applied for both in vitro and in vivo generated samples.

ADC PK in Nonclinical Species. Since ADCs, by design, use internalization of the ADC-receptor complex as the mechanism for the drug's delivery, cross-reactivity of the carrier mAb to the target in nonclinical species would affect the ADC's PK and distribution. The binding affinities of the ADC to the target in multiple species are typically measured during the early stages of drug discovery. If the antibody is not cross-reactive to the rodent target, the PK and toxicity may not be reflective of PK or toxicity in a target-expressing species. However, it may still provide some information on the nonspecific disposition of ADCs and on potential drug-related metabolites (Kamath and Iyer, 2015). The choice of animal species to evaluate the PK of an ADC incorporating a novel antibody typically follows the same general principles as an unconjugated antibody (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH S6). In addition, species selection for a novel drug incorporates considerations used for a new chemical entity (for anticancer products in accordance with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use ICH S9, (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm085389.pdf) on a case-by case basis, based on the mode of action of the drug. In most cases, the PK of an ADC is characterized at doses low enough to evaluate target-mediated clearance and at doses high enough to understand toxicokinetics. TAb, ADC, and released drug are typically quantified over the study duration. ADCs are usually administered by the intravenous route, thus obviating the absorption phase. Although the TAb and ADC systemic concentration time profiles are those normally observed after intravenous administration, the profile of the released drug, akin to the exposure profile of the active component of a prodrug typically resembles that of an extravascularly administered compound.

ADC Tissue Distribution. A general determination of whole-body tissue distribution of the ADC can be considered to determine distribution between target-expressing and nontarget-expressing tissues. The distribution of an ADC in various tissues and subsequent deconjugation or catabolism to release the drug affects its efficacy and safety (Alley et al., 2009; Boswell et al., 2011). In addition, information on active drug metabolites in the tissues can also be obtained, which may confer additional activity. Tissue distribution studies can be conducted in rodents (rats and/or tumor-bearing mice) to evaluate distribution to normal tissues (or tumor). These types of studies are typically conducted with radiolabeled ADCs, in which the radiolabel could be applied on the drug (usually C-14 or H-3) or simultaneously on both the antibody and drug using a dual-labeled ADC with C-14 and H-3 (Alley et al., 2009). Although evaluation of the whole-body tissue distribution in rodents using radiolabeled ADC can be considered, this assessment may not always be appropriate because of challenging and expensive synthesis, limitations in sensitivity and resolution of this technique, as well as typical lack of cross-reactivity to rodent targets.

ADME (Mass Balance) Evaluation. A human ADME study using radiolabeled material is not currently recommended for the following reasons. For the cytotoxic/genotoxic drugs typically used in oncology ADCs, dosing of ADCs in healthy volunteers is not appropriate. Therefore, such an evaluation would have to be conducted in patients with cancer. Because of the typically long ADC half-life, patients would have to be sequestered for prolonged periods of time (3 to 4 weeks) with little to no benefit to the patient, which would not be ethical. An ADME study of shorter duration may not be adequate and can result in incomplete mass balance data. In addition, identification of the circulating products of further metabolism of the drug may be challenging because of typically very low concentrations of those products. Therefore, a traditional human ADME study for an ADC is

not feasible. An animal (rodent) ADME study using an ADC with radiolabel on the drug may be considered instead (Erickson and Lambert, 2012). Various matrices such as bile (using bile-duct cannulated rats), urine, and feces can be collected in addition to serum/plasma. This evaluation could help to understand the metabolism and excretion routes of an ADC and released drug (or drug-containing species). However, it should be noted that since most of the ADCs do not cross-react with rodent targets, this evaluation would primarily address nonspecific uptake and degradation pathways and may not necessarily represent the disposition of ADC in humans. In addition, because of the long half-life of ADCs, the study duration would need to be extended to achieve good recovery of radioactivity and mass balance.

Novel ADCs with Previously Characterized Drugs. Drugs or linker drugs that have been previously tested in the clinic can be conjugated to different mAbs to form new ADCs. In these cases, some of the ADME information can be obtained from existing published reports/filings and/or internally garnered unpublished data, and evaluation would focus on generating key data specific to the novel ADC. Often, a well studied drug is conjugated to a mAb via a novel linker sequence or using unreported conjugation chemistries (i.e., site-specific relative to conventional cysteine or lysine residue based conjugation chemistry). Therefore, ADME evaluation would address major released drug-containing species, plasma stability of the ADC, and major ADC clearance mechanisms and would confirm that projected human PK properties support the intended dose and frequency of administration.

Release of the Drug from an ADC

The drug is intended to be released intracellularly in the target tissue, in most cases via proteolytic cleavage of the linker or catabolism of the entire ADC molecule in lysosomes. However, current ADCs are not completely stable in the circulation and nonspecific release of the drug or transfer of the drug to other serum components has been reported (Alley et al., 2008). Understanding the mechanism by which the drug is released from the ADC helps to identify potential pharmacologically active drug-containing products and select the appropriate bioanalytical methods. This evaluation involves identification and quantitation of the major released drug-containing species. In addition, drug might be cleaved by extracellular proteases, especially in the proximity of the tumor. Therefore, understanding the cellular permeability of drug-containing products can help understand the pharmacodynamics and potential bystander effects of ADCs.

Experimental Systems for In Vitro Assessment of Drug Release from an ADC. In general, in vitro systems that can be used for identification of drug-containing species released from an ADC as well as products of their further metabolism are similar to those used for traditional small molecule drug metabolism studies. However, specific experimental conditions might need to be adjusted to accommodate unique aspects of an ADC's properties.

Similar to traditional mAbs, tissue distribution of the ADC is low, and the majority of the ADC distributes to the organs where IgG catabolism takes place, with the liver playing a prominent role in ADC clearance (Boswell et al., 2011; Shen et al., 2012a). Therefore, one would expect that the majority of ADC catabolism as well as linker and drug metabolism might occur in the liver. Hepatocytes are the most complete system that contains all relevant microsomal enzymes as well as cytosolic enzymes, such as aldehyde oxidase, peptidases, and so forth. However, because of the lack of target protein expression on hepatocytes, utilizing this system for studying drug release from ADCs is limited. In addition, using hepatocytes for evaluation of the released drug's metabolism may be limited by its permeability. Liver microsomes are a convenient in vitro system that contains cytochrome P450 (P450) and UDP-glucuronosyltransferase enzymes and are not confounded by the drug's permeability, uptake, or toxicity. However, liver microsomes lack the cytosolic and lysosomal enzymes that, in many cases, are responsible for the release of the drug from the ADC molecule. Therefore, liver microsomes could be considered a good tool for studying the metabolic pathways of the released drug but may have limited utility for studying the drug release from ADC molecule.

Since ADCs have been primarily used in the treatment of cancer, cancer cells could potentially be used as a system for studying drug release from the ADC (Erickson et al., 2012). However, selection of the appropriate cell line would depend on the target expression, to facilitate target-mediated uptake of ADC by the cells; therefore, it cannot be standardized and used across multiple programs. In general, although cancer cells express some drug-metabolizing enzymes, all of those enzymes are found in the liver as well. Moreover, cancer cells have been shown to upregulate phase II enzymes and downregulate phase I enzymes compared with the liver (Rodríguez-Antona et al., 2002; Zahreddine and Borden, 2015).

Lysosomal preparations represent another potential in vitro system. Although lysosomes can be used to study the release of the drug from the ADC, because they mimic ADC degradation in the cell, they are an artificial system that does not contain drug-metabolizing enzymes such as P450s or UDP-glucuronosyltransferases. Therefore, lysosomes cannot be used for metabolism studies of the drug itself. In addition, uptake of the ADC into the lysosomes might be limited, which may hamper the stability assessment.

The liver S9 fraction contains all major drug-metabolizing enzymes, does not rely on the permeability of the drug, is transporter independent, and is less susceptible to cytotoxic agents. In addition, the S9 fraction can be used at either pH 7.4 (to study metabolism of the drug) or acidified to mimic the pH of the lysosomal environment, which is the site of degradation of an ADC. Therefore, this system can be used for studying drug release and profiling of drug-containing species of both an intact ADC and drug.

In general, it is recommended that understanding of the linker and drug chemical structures and potential reactions that they can undergo be taken into consideration when selecting the in vitro test system and the most straightforward (or simplest) system is used.

DDI Potential. Based on the information from the limited number of ADCs in the clinic, their potential for DDI is typically considered to be low. However, since first-in-human clinical studies with ADCs are typically conducted in patients who also take multiple concomitant medications, it is useful to assess the DDI risk of the released drug at the preclinical stage based on its ADME characteristics. The drug released from an ADC can be eliminated unchanged or metabolized by enzymes such as the P450 system (Fig. 2). Direct renal or biliary elimination could potentially be a significant component of the overall drug's clearance. DDIs are quite common for small molecule drugs, mainly owing to inhibition or induction of drug-metabolizing enzymes and transporters. In most cases, systemic concentrations of the released drug are extremely low; therefore, the risk of the ADC being a DDI perpetrator can be considered minimal (Han and Zhao, 2014). However, one might expect the liver to receive higher concentrations of the drug, as part of nonspecific catabolic clearance of an ADC, than those inferred from drug's systemic concentration. Nevertheless, in a clinical DDI study, Adcetris (brentuximab vedotin, a valine-citrulline dipeptide MMAE ADC) did not affect the PK of midazolam (CYP3A substrate) (Adcetris drug label, (http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/125388s000,125399s000lbl.pdf). Investigation of a novel drug as an enzyme inhibitor or inducer should be conducted in accordance with the most current version of U.S. Food and Drug Administration guidelines (2012 Draft Guidance for Industry;

(http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM194490.pdf) and European Medicines Agency guidelines (2015 Guideline on Investigation of Drug Interactions; (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf).

In general, the probability of a released drug to be a DDI victim exists and impact can be high because these cytotoxic drugs typically have a narrow therapeutic margin. Therefore, inhibition of their clearance might lead to an increase in drug exposure in tissues and in the circulation, which could result in toxicities. When coadministered with rifampicin (CYP3A inducer) and ketoconazole (CYP3A inhibitor), no changes in the PK of Adcetris were observed. However, exposure of released MMAE was reduced by approximately 46% and increased by approximately 34% by coadministration of rifampicin and ketoconazole, respectively (Adcetris drug label: (http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/125388s000,125399s000lbl.pdf). In addition, although no formal DDI studies have been conducted with Kadcyla, a DM1-containing ADC, its label (http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125427lbl.pdf) contains a caution that coadministration with strong CYP3A4 inhibitors should be avoided because of the potential for an increase in DM1 exposure and toxicity. Because most of the patients will also take a number of concomitant medications, a DDI risk assessment for the ADC including in vitro evaluation of enzyme interactions (in particular, reaction phenotyping for P450 metabolism) for drug and potential major circulating drug metabolites need to be performed during development to determine whether formal clinical studies should be conducted. Studies to assess transporter-mediated DDIs may be valuable at later stages of the development.

Considerations for the Released Drug. Potentially, the drug can undergo further metabolism upon release, which can affect the observed toxicity and pharmacology of the ADC. Understanding the mechanism and identification of the metabolites may provide insight into drug-related species to monitor in subsequent animal and human studies. However, systemic concentrations of these species are generally low; therefore, there may be no need, or insufficient assay sensitivity may not allow the detection of them. In vivo samples obtained from high-dose toxicity animals might be the best place to look for such products. If deemed necessary, based on in vitro or animal in vivo data, identification of circulating products of further metabolism of the drug may be performed in patients using unlabeled ADC.

Information on plasma protein binding and permeability of the drug can be used for understanding ADC's off-target toxicity because of the released drug's distribution into cells/tissues by active uptake or passive diffusion, rather than for understanding the ADC's pharmacological activity as it is driven by the drug released inside the target cells.

For novel drugs, a PK study in rodents after an intravenous administration of the unlabeled unconjugated drug should be conducted. The dose is typically selected based on the total conjugated drug load at the ADC dose, which is expected to be below the maximum tolerated dose. In vivo metabolite scouting can be included in the study design (http://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm292362.pdf). Collection of urine and bile can also be incorporated into this evaluation; however, sensitivity may limit the utility of these data.

Conclusions

ADME characterization for an ADC is a complex process because it needs to take into account both the mAb and small molecule components of this modality. Although no standard "one-size-fits-all" approach can be applied to all ADCs, this review outlines the advantages and disadvantages of the currently used experimental systems and strategies and provides guidance that should help investigators to develop successful novel ADCs with desirable ADME properties. Since ADC technology is still evolving, there needs to be a continuous re-evaluation of ADME approaches as it matures over the next several years.

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